IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Olli Vuolteenaho et al.	Confirmation No.:	9413
Serial No.:	10/562,081	Art Unit:	1647
Filed:	April 5, 2006	Examiner:	Shulamith H. Shafer
Customer No.:	21559		
Title:	Methods of Determination of Activation or Inactivation of Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP) Hormonal Systems		

DECLARATION OF PROFESSOR OLLI VUOLTEENAHO AND PROFESSOR HEIKKI RUSKOAHO UNDER 35 U.S.C. § 1.132

We, Professors Olli Vuolteenaho and Heikki Ruskoaho, declare:

1. We are two of the five co-inventors of the subject matter described and claimed in the above-captioned patent application. We are professors at the University of Oulu, in Oulu Finland. We are experts in the field of natriuretic peptides, and together have more than three hundred publications in this field.

2. We have read and we understand the Office Action dated July 9, 2010 in connection with the application.

3. The natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), form a very complex regulatory system that the body uses to protect the heart from overloading. Despite the very different regulation of the formation and secretion of proANP- and proBNP-derived peptides, the biological information conveyed by these peptides converges in target cells via a common receptor, Natriuretic Peptide Receptor-A (NPR-A, also known as guanylyl cyclase-A or GC-A).

4. There is significant basal secretion of proANP-derived peptides, whereas proBNP-derived peptides are formed and secreted on demand. Moreover, BNP has a lower affinity to NPR-A as compared to ANP. Thus, small absolute increases of BNP do not induce significant biological effects. A pure BNP or N-terminal fragment of pro-BNP (NT-proBNP) assay detects

them as proportionally large increases, because of the negligible basal secretion of proBNP peptides. On the other hand, relatively small proportional increases of proANP-derived peptides induce marked biological effects. Concomitant increase of both proANP- and proBNP-derived peptides, even when modest, indicates a major pathophysiological induction of the cardiac natriuretic peptides.

5. Measurement of proANP- and proBNP-derived peptides with separate assays requires the optimization, calibration, and maintenance of at least two immunoassays, with resulting technical complexity and high cost. Use of two assays also requires sophisticated software for extraction of clinically relevant information from the non-linear data from the two assays. The results from the separate assays reported to the physician would then consist of two analytical values and their mathematical interpretation, and would not be very easily understandable or intuitive. Thus, routine measurement of proANP- and proBNP-derived peptides is not a practical or viable alternative for routine clinical application.

6. We devised a novel method which detects activation or inactivation of the ANP and BNP hormonal systems by assaying for both proANP- and proBNP- derived peptides in the same sample, at the same time, in a single reading, in a single assay. Practical tests of our present invention showed that it does not simply replicate the function of two separate assays, a result which would have made the invention useless. Rather, it appears to circumvent the above-described pitfalls associated with the use of separate assays, because it mimics more closely the way the body processes the biological information carried by the cardiac natriuretic peptides. The method produces a single result, does not require sophisticated data extraction, and is simpler to perform than prior art methods. Small increases of only NT-proBNP are masked by the high basal levels of NT-proANP, thus decreasing the risk of false positive results. On the other hand, even small increases of both NT-proANP and NT-proBNP can be detected with high sensitivity, thus decreasing the risk of false negative results. Thus, the technical innovation of the present invention provides a superior clinical method without increasing the technical complexity or cost.

7. These results are not self-evident and could not have been predicted with prior knowledge without devising the novel method and testing it with actual clinical samples. This is

illustrated by a practical example in Figure 4/5 of the present application, in which clinical samples of 500 patients with heart failure were measured. Unexpectedly the NT-proXNP value (Figure 4/5, bar on the right) provides a clearly better separation between the different New York Heart Association (NYHA) classes, and thus has more clinical power, as compared to NT-proANP alone (bar on the left), NT-proBNP alone (bar in the middle), or their arithmetic sum, measured from the same samples.

8. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: May 2, 2011

Professor Olli Vuolteenaho

Date: May 2, 2011

Professor Heikki Ruskoaho